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 NEWS  4  Feb 16  TOXLINE no longer being updated
 NEWS  5  Apr 23  Search Derwent WPINDEX by chemical structure
 NEWS  6  Apr 23  PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA
 NEWS  7  May 07  DGENE Reload

 NEWS EXPRESS  May 23 CURRENT WINDOWS VERSION IS V6.0a,
               CURRENT MACINTOSH VERSION IS V5.0C (ENG) AND V5.0JB (JP),
               AND CURRENT DISCOVER FILE IS DATED 06 APRIL 2001
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=> s (antibody or immunoglobulin) (P) (hydrophilicity or solubul? or yield or recovery or expression or increas?)
   3 FILES SEARCHED...
L1      505481 (ANTIBODY OR IMMUNOGLOBULIN) (P) (HYDROPHILICITY OR SOLUBUL? OR
               YIELD OR RECOVERY OR EXPRESSION OR INCREAS?)

=> s l1 (P) (domain or fragment)
L2       47308 L1 (P) (DOMAIN OR FRAGMENT)

=> s l2 (P) DNA
L3        7807 L2 (P) DNA

=> s l3 (P) interface
L4          17 L3 (P) INTERFACE

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5           6 DUP REM L4 (11 DUPLICATES REMOVED)

=> dis l5 1-6 kwic ibib abs

L5   ANSWER 1 OF 6  CAPLUS  COPYRIGHT 2001 ACS
AB   The present invention relates to the modification of Ig
     superfamily (IgSF) domains, IgSF fragments and fusion
     proteins thereof, esp. to the modification of antibody derivs.,
     so as to improve their soly., and hence the yield, and ease of
     handling.  The inventors have found that this can be achieved by making
     the region which comprises the interface with domains
     adjoined to said IgSF domain in a larger fragment or a
     full IgSF protein, and which becomes exposed in the IgSF domain,
     more hydrophilic by modification.  The present invention describes
     DNA sequences encoding modified IgSF domains or
     fragments and fusion proteins thereof, vectors and hosts contg.
     these DNA sequences, IgSF domains or fragments
     or fusion proteins obtainable by expressing said DNA sequences
     in suitable expression systems, and a method for modifying IgSF
     domains, so as to improve their soly., expressibility and ease of
     handling.
ACCESSION NUMBER:         1998:71159  CAPLUS
DOCUMENT NUMBER:          128:139760
TITLE:                    Immunoglobulin superfamily domains and fragments with
                          increased solubility
INVENTOR(S):              Pluckthun, Andreas; Nieba, Lars; Honegger, Annemarie
PATENT ASSIGNEE(S):       Morphosys Gesellschaft Fur Proteinoptimierung M.b.H.,
                          Germany; Pluckthun, Andreas; Nieba, Lars; Honegger,
                          Annemarie
SOURCE:                   PCT Int. Appl., 61 pp.
```

CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9802462	A1	19980122	WO 1997-EP3792	19970716
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 938506	A1	19990901	EP 1997-934467	19970716
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000516452	T2	20001212	JP 1998-505618	19970716

PRIORITY APPLN. INFO.: EP 1996-111441 A 19960716
WO 1997-EP3792 W 19970716

AB The present invention relates to the modification of **Ig** superfamily (IgSF) **domains**, IgSF **fragments** and fusion proteins thereof, esp. to the modification of **antibody** derivs., so as to improve their soly., and hence the **yield**, and ease of handling. The inventors have found that this can be achieved by making the region which comprises the **interface** with **domains** adjoined to said IgSF **domain** in a larger **fragment** or a full IgSF protein, and which becomes exposed in the IgSF **domain**, more hydrophilic by modification. The present invention describes **DNA** sequences encoding modified IgSF **domains** or **fragments** and fusion proteins thereof, vectors and hosts contg. these **DNA** sequences, IgSF **domains** or **fragments** or fusion proteins obtainable by expressing said **DNA** sequences in suitable **expression** systems, and a method for modifying IgSF **domains**, so as to improve their soly., expressibility and ease of handling.

L5 ANSWER 2 OF 6 MEDLINE DUPLICATE 1

AB . . . beta and transforming growth factor-beta (TGF beta) in regulating TIMP-1, TIMP-3, and 92-kDa type IV collagenase messenger ribonucleic acid (mRNA) **expression** in human endometrial stromal cells using quantitative competitive PCR. Confluent stromal cell cultures treated with progesterone and estradiol for 9 days were stimulated with IL-1 beta, IL-1 beta plus anti-IL-1 beta **antibody**, TGF beta, and TGF beta plus anti-TGF beta **antibody** for an additional 24 h. Competitive complementary **DNA fragments** were constructed by deletion of a defined **fragment** from each of the target complementary **DNA** sequences and coamplified in quantitative competitive PCR as an internal standard. TIMP-1 and TIMP-3, but not 92-kDa type IV collagenase. . . . IV collagenase mRNA was only expressed after stimulation with IL-1 beta. IL-1 beta both augmented 92-kDa type IV collagenase mRNA **expression** and decreased TIMP-1 and TIMP-3 mRNA **expression** in a dose-dependent manner. Conversely, TGF beta augmented TIMP-1 and TIMP-3 mRNA **expression**, but did not affect 92-kDa type IV collagenase **expression**. IL-1 and TGF beta-mediated changes were both neutralized by specific **antibodies** . These results provide indirect evidence that IL-1 and TGF beta may play crucial roles at the embryo-maternal **interface** during trophoblast invasion by regulating stromal cell **expression** of TIMP-1, TIMP-3, and 92-kDa type IV collagenase, all of which are known to be important in trophoblast invasion.

ACCESSION NUMBER: 1998251593 MEDLINE
DOCUMENT NUMBER: 98251593 PubMed ID: 9589682
TITLE: Cytokine-mediated regulation of 92-kilodalton type IV collagenase, tissue inhibitor or metalloproteinase-1 (TIMP-1), and TIMP-3 messenger ribonucleic acid expression in human endometrial stromal cells.
AUTHOR: Huang H Y; Wen Y; Irwin J C; Kruessel J S; Soong Y K; Polan M L
CORPORATE SOURCE: Department of Gynecology and Obstetrics, Stanford University Medical Center and School of Medicine, California 94305, USA.
CONTRACT NUMBER: HD-31575 (NICHD)
SOURCE: JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, (1998 May) 83 (5) 1721-9.
Journal code: HRB; 0375362. ISSN: 0021-972X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199806
ENTRY DATE: Entered STN: 19980611
Last Updated on STN: 20000303
Entered Medline: 19980604

AB Interleukin-1 (IL-1) is expressed in human endometrium and has been shown to play an integral role in local cellular interactions during implantation. In addition, the matrix metalloproteinase (MMP) and its inhibitor, the tissue inhibitor of metalloproteinase (TIMP), are crucial during implantation, mediating in vitro trophoblast penetration, and are regulated by several cytokines expressed by trophoblast cells. We have investigated the roles of IL-1 beta and transforming growth factor-beta (TGF beta) in regulating TIMP-1, TIMP-3, and 92-kDa type IV collagenase messenger ribonucleic acid (mRNA) **expression** in human endometrial stromal cells using quantitative competitive PCR. Confluent stromal cell cultures treated with progesterone and estradiol for 9 days were stimulated with IL-1 beta, IL-1 beta plus anti-IL-1 beta **antibody**, TGF beta, and TGF beta plus anti-TGF beta **antibody** for an additional 24 h. Competitive complementary **DNA fragments** were constructed by deletion of a defined **fragment** from each of the target complementary DNA sequences and coamplified in quantitative competitive PCR as an internal standard. TIMP-1 and TIMP-3, but not 92-kDa type IV collagenase mRNA, were expressed in stromal cells. The 92-kDa type IV collagenase mRNA was only expressed after stimulation with IL-1 beta. IL-1 beta both augmented 92-kDa type IV collagenase mRNA **expression** and decreased TIMP-1 and TIMP-3 mRNA **expression** in a dose-dependent manner. Conversely, TGF beta augmented TIMP-1 and TIMP-3 mRNA **expression** , but did not affect 92-kDa type IV collagenase **expression**. IL-1 and TGF beta-mediated changes were both neutralized by specific **antibodies**. These results provide indirect evidence that IL-1 and TGF beta may play crucial roles at the embryo-maternal **interface** during trophoblast invasion by regulating stromal cell **expression** of TIMP-1, TIMP-3, and 92-kDa type IV collagenase, all of which are known to be important in trophoblast invasion.

L5 ANSWER 3 OF 6 MEDLINE DUPLICATE 2

AB The monoclonal **antibody**, UB25, recognises a glycoprotein specifically located at the biotrophic **interface** formed in the Colletotrichum lindemuthianum-bean interaction. The **antibody** labels the walls of intracellular hyphae and the interfacial matrix which separates them from the invaginated host plasma membrane. In. . . are multiples of M(r) 40.5 kDa. A full length cDNA encoding the glycoprotein recognised by UB25 has been isolated by **expression** cloning and designated CIH1 (Colletotrichum Intracellular Hypha 1). In vitro transcription/translation of CIH1, and transfection of mammalian COS cells, showed. . . product in both procedures confirming that the clones isolated were true positives. Southern analysis of bean and C. lindemuthianum genomic **DNA** indicated that the CIH1 glycoprotein is fungally encoded and Northern analysis showed that it is only expressed in planta. Analysis. . . acid sequence of CIH1 indicates the presence of an N-terminal signal sequence and two possible sites for N-glycosylation. The N-terminal **domain** of the mature protein is rich in proline and contains several short repetitive motifs. CIH1 is thus a fungal proline-rich. . .

ACCESSION NUMBER: 1998388657 MEDLINE
DOCUMENT NUMBER: 98388657 PubMed ID: 9721685
TITLE: Expression cloning of a fungal proline-rich glycoprotein specific to the biotrophic interface formed in the Colletotrichum-bean interaction.
AUTHOR: Perfect S E; O'Connell R J; Green E F; Doering-Saad C; Green J R
CORPORATE SOURCE: School of Biological Sciences, University of Birmingham, UK.
SOURCE: PLANT JOURNAL, (1998 Jul) 15 (2) 273-9.
Journal code: BRU; 9207397. ISSN: 0960-7412.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AJ001441
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981020
Last Updated on STN: 20000303
Entered Medline: 19981005

AB The monoclonal **antibody**, UB25, recognises a glycoprotein specifically located at the biotrophic **interface** formed in the Colletotrichum lindemuthianum-bean interaction. The **antibody** labels the walls of intracellular hyphae and the interfacial matrix which separates them from the invaginated host plasma membrane. In Western blots, UB25 recognises a ladder of bands which are multiples of M(r) 40.5 kDa. A full length cDNA encoding the glycoprotein recognised by UB25 has been isolated by **expression** cloning and designated CIH1 (Colletotrichum Intracellular Hypha 1). In vitro transcription/translation of CIH1, and transfection of mammalian COS cells, showed that UB25 recognized the expressed product in both procedures confirming that the clones isolated were true positives. Southern analysis of bean and C. lindemuthianum genomic **DNA** indicated that the CIH1 glycoprotein is fungally encoded and Northern analysis showed that it is only expressed in planta. Analysis of the deduced amino acid sequence of CIH1 indicates the presence of an N-terminal signal sequence and two possible sites for N-glycosylation. The N-terminal **domain** of the mature protein is rich in proline and contains several short repetitive motifs. CIH1 is thus a fungal proline-rich glycoprotein which appears to form a cross-linked structure in planta and, as such, resembles plant cell wall proline- and hydroxyproline-rich proteins. Possible functions for the CIH1 protein in the establishment and maintenance of biotrophy are discussed.

L5 ANSWER 4 OF 6 MEDLINE DUPLICATE 3

AB . . . ER during fetal uterine development. The purpose of this study was to investigate the pattern of ER gene and protein **expression** in the human fetal uterus. Uteri were obtained from abortuses (n = 43; range, 10-24 weeks gestation) of women undergoing. . . cultured fetal uterine stroma-like cells (n = 15). Immunolocalization studies were performed on frozen uterine sections using the H222 monoclonal **antibody** (Abbott Laboratories) directed against the ER (n = 20). Western blotting was used to confirm the identity of the ER. . . = 3). Ligand binding studies were performed using radiolabeled [3H]estradiol (n = 5). Using reverse transcription-polymerase chain reaction, a 263-basepair **DNA fragment** corresponding to the ER was consistently present in uteri after 15 weeks gestation. By immunohistochemistry, the ER is expressed within the uterine mesenchyme in a discrete cylindrical pattern at the **interface** of the differentiating endometrial stroma and myometrium. Western blotting confirmed the presence of a protein of an apparent mol wt. . .

ACCESSION NUMBER: 95189894 MEDLINE
DOCUMENT NUMBER: 95189894 PubMed ID: 7883857
TITLE: Ontogeny of the estrogen receptor in the human fetal uterus.
AUTHOR: Glatstein I Z; Yeh J
CORPORATE SOURCE: Department of Obstetrics, Gynecology, and Reproductive Biology, Brigham and Women's Hospital, Boston, Massachusetts 02115.
SOURCE: JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM, (1995 Mar) 80 (3) 958-64.
Journal code: HRB; 0375362. ISSN: 0021-972X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199504
ENTRY DATE: Entered STN: 19950425
Last Updated on STN: 19950425
Entered Medline: 19950411

AB The role of estrogen and its receptor in the development of the human fetal reproductive tract is unknown, but it may be involved in uterine maturation. In mouse and guinea pig uteri, studies have identified estrogen receptor (ER) protein during the fetal period. In the human, there are no published data regarding the ER during fetal uterine development. The purpose of this study was to investigate the pattern of ER gene and protein **expression** in the human fetal uterus. Uteri were obtained from abortuses (n = 43; range, 10-24 weeks gestation) of women undergoing elective termination of pregnancy. Reverse transcription-polymerase chain reaction was performed with whole uteri as well as cultured fetal uterine stroma-like cells (n = 15). Immunolocalization studies were performed on frozen uterine sections using the H222 monoclonal **antibody** (Abbott Laboratories) directed against the ER (n = 20). Western blotting was used to confirm the identity of the ER protein (n = 3). Ligand binding studies were performed using radiolabeled [3H]estradiol (n = 5). Using reverse transcription-polymerase

chain reaction, a 263-basepair DNA fragment corresponding to the ER was consistently present in uteri after 15 weeks gestation. By immunohistochemistry, the ER is expressed within the uterine mesenchyme in a discrete cylindrical pattern at the **interface** of the differentiating endometrial stroma and myometrium. Western blotting confirmed the presence of a protein of an apparent mol wt of 66 kilodaltons, the predicted size of the ER. Ligand binding studies for the ER gave a value less than 8 fmol/mg protein. In summary, the ER gene is expressed in the uterus beginning in the early second trimester during fetal development. ER protein is localized in a discrete cylindrical pattern within the developing uterus. The highly specific location of the ER protein together with the messenger ribonucleic acid data suggest a role for the ER in differentiation of the primitive uterine mesenchyme into stromal and myometrial compartments.

L5 ANSWER 5 OF 6 MEDLINE DUPLICATE 4

AB . . . **adhesion** molecule-1 (ELAM-1), a cell surface glycoprotein expressed by cytokine-activated endothelium, mediates the adhesion of blood neutrophils. A full-length complementary **DNA** (cDNA) for ELAM-1 has now been isolated by transient **expression** in COS cells. Cells transfected with the ELAM-1 clone express a surface structure recognized by two ELAM-1 specific monoclonal **antibodies** (H4/18 and H18/7) and support the adhesion of isolated human neutrophils and the promyelocytic cell line HL-60. **Expression** of ELAM-1 transcripts in cultured human endothelial cells is induced by cytokines, reaching a maximum at 2 to 4 hours and decaying by 24 hours; cell surface **expression** of ELAM-1 protein parallels that of the mRNA. The primary sequence of ELAM-1 predicts an amino-terminal lectin-like **domain**, an EGF **domain**, and six tandem repetitive motifs (about 60 amino acids each) related to those found in complement regulatory proteins. A similar **domain** structure is also found in the MEL-14 lymphocyte cell surface homing receptor, and in granule-membrane protein 140, a membrane glycoprotein. . . . of a nascent gene family of cell surface molecules involved in the regulation of inflammatory and immunological events at the **interface** of vessel wall and blood.

ACCESSION NUMBER: 89162047 MEDLINE
DOCUMENT NUMBER: 89162047 PubMed ID: 2466335
TITLE: Endothelial leukocyte adhesion molecule 1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins.
AUTHOR: Bevilacqua M P; Stengelin S; Gimbrone M A Jr; Seed B
CORPORATE SOURCE: Department of Pathology, Brigham and Women's Hospital, Boston, MA 02115.
CONTRACT NUMBER: PO1 HL-36028 (NHLBI)
SOURCE: SCIENCE, (1989 Mar 3) 243 (4895) 1160-5.
Journal code: UJ7; 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M24736
ENTRY MONTH: 198904
ENTRY DATE: Entered STN: 19900306
Last Updated on STN: 19970203
Entered Medline: 19890407

AB Focal adhesion of leukocytes to the blood vessel lining is a key step in inflammation and certain vascular disease processes. Endothelial leukocyte adhesion molecule-1 (ELAM-1), a cell surface glycoprotein expressed by cytokine-activated endothelium, mediates the adhesion of blood neutrophils. A full-length complementary **DNA** (cDNA) for ELAM-1 has now been isolated by transient **expression** in COS cells. Cells transfected with the ELAM-1 clone express a surface structure recognized by two ELAM-1 specific monoclonal **antibodies** (H4/18 and H18/7) and support the adhesion of isolated human neutrophils and the promyelocytic cell line HL-60. **Expression** of ELAM-1 transcripts in cultured human endothelial cells is induced by cytokines, reaching a maximum at 2 to 4 hours and decaying by 24 hours; cell surface **expression** of ELAM-1 protein parallels that of the mRNA. The primary sequence of ELAM-1 predicts an amino-terminal lectin-like **domain**, an EGF **domain**, and six tandem repetitive motifs (about 60 amino acids each) related to those found in complement regulatory proteins. A similar **domain** structure is also found in the MEL-14 lymphocyte cell surface homing receptor, and in granule-membrane protein 140, a membrane glycoprotein of platelet and endothelial secretory granules that can be rapidly mobilized (less than 5 minutes) to the cell surface by thrombin and other stimuli. Thus, ELAM-1 may be a member of a nascent gene family of cell surface molecules involved in the regulation of inflammatory and immunological events at the **interface** of vessel wall and blood.

L5 ANSWER 6 OF 6 MEDLINE DUPLICATE 5

AB . . . The starting model was the refined 2.7 A structure of unliganded Fab from an autoantibody (BV04-01) with specificity for single-stranded **DNA**. In the 4-4-20 complex fluorescein fits tightly into a relatively deep slot formed by a network of tryptophan and tyrosine. . . chains. The planar xanthonyl ring of the hapten is accommodated at the bottom of the slot while the phenylcarboxyl group **interfaces** with solvent. Tyrosine 37 (light chain) and tryptophan 33 (heavy chain) flank the xanthonyl group and tryptophan 101 (light chain). . . . haptenic dianion. Formation of an enol-arginine ion pair in a region of low dielectric constant may account for an incremental **increase** in affinity of 2-3 orders of magnitude in the 4-4-20 molecule relative to other members of an idiotypic family of monoclonal antifluorescyl **antibodies**. The phenyl carboxyl group of fluorescein appears to be hydrogen bonded to the phenolic hydroxyl group of tyrosine 37 of the light chain. A molecule of 2-methyl-2,4-pentanediol (MPD), trapped in the **interface** of the variable **domains** just below the fluorescein binding site, may be partly responsible for the decrease in affinity for the hapten in MPD.

ACCESSION NUMBER: 90017460 MEDLINE
DOCUMENT NUMBER: 90017460 PubMed ID: 2508085
TITLE: Three-dimensional structure of a fluorescein-Fab complex crystallized in 2-methyl-2,4-pentanediol.
AUTHOR: Herron J N; He X M; Mason M L; Voss E W Jr; Edmundson A B
CORPORATE SOURCE: Department of Biology, University of Utah, Salt Lake City 84112.
CONTRACT NUMBER: AI 20960 (NIAID)
AI 22898 (NIAID)
CA 19616 (NCI)
SOURCE: PROTEINS, (1989) 5 (4) 271-80.
Journal code: PTS; 8700181. ISSN: 0887-3585.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198911
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19980206
Entered Medline: 19891108

AB The crystal structure of a fluorescein-Fab (4-4-20) complex was determined at 2.7 A resolution by molecular replacement methods. The starting model was the refined 2.7 A structure of unliganded Fab from an autoantibody (BV04-01) with specificity for single-stranded **DNA**. In the 4-4-20 complex fluorescein fits tightly into a relatively deep slot formed by a network of tryptophan and tyrosine side chains. The planar xanthonyl ring of the hapten is accommodated at the bottom of the slot while the phenylcarboxyl group **interfaces** with solvent. Tyrosine 37 (light chain) and tryptophan 33 (heavy chain) flank the xanthonyl group and tryptophan 101 (light chain) provides the floor of the combining site. Tyrosine 103 (heavy chain) is situated near the phenyl ring of the hapten and tyrosine 102 (heavy chain) forms part of the boundary of the slot. Histidine 31 and arginine 39 of the light chain are located in positions adjacent to the two enolic groups at opposite ends of the xanthonyl ring, and thus account for neutralization of one of two negative charges in the haptenic dianion. Formation of an enol-arginine ion pair in a region of low dielectric constant may account for an incremental **increase** in affinity of 2-3 orders of magnitude in the 4-4-20 molecule relative to other members of an idiotypic family of monoclonal antifluorescyl **antibodies**. The phenyl carboxyl group of fluorescein appears to be hydrogen bonded to the phenolic hydroxyl group of tyrosine 37 of the light chain. A molecule of 2-methyl-2,4-pentanediol (MPD), trapped in the **interface** of the variable **domains** just below the fluorescein binding site, may be partly responsible for the decrease in affinity for the hapten in MPD.

```
=> s Pluckthun A?au or Nieba L?au or Honegger A?/au
'?' TRUNCATION SYMBOL NOT VALID WITHIN 'A?AU'
'?' TRUNCATION SYMBOL NOT VALID WITHIN 'A?AU'
'?' TRUNCATION SYMBOL NOT VALID WITHIN 'A?AU'
'?' TRUNCATION SYMBOL NOT VALID WITHIN 'A?AU'
The truncation symbol ? may be used only at the end of a search
term. To specify a variable character within a word use '!', e.g.,
'wom!n' to search for both 'woman' and 'women'. Enter "HELP
TRUNCATION" at an arrow prompt (=>) for more information.

=> s Pluckthun A?/au or Nieba L?/au or Honegger A?/au
L6          651 PLUCKTHUN A?/AU OR NIEBA L?/AU OR HONEGGER A?/AU

=> s l6 and antibod?
L7          313 L6 AND ANTIBOD?

=> s l7 and hydro?
L8           46 L7 AND HYDRO?

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9           20 DUP REM L8 (26 DUPLICATES REMOVED)

=> dus l9 1-20 ibib abs
DUS IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> dis l9 1-20 ibib abs

L9   ANSWER 1 OF 20  MEDLINE
ACCESSION NUMBER:    2001150051      MEDLINE
DOCUMENT NUMBER:     21103695   PubMed ID: 11162118
TITLE:               The scFv fragment of the antibody hu4D5-8:
                     evidence for early premature domain interaction in
                     refolding.
AUTHOR:              Jager M; Gehrig P; Pluckthun A
CORPORATE SOURCE:    Biochemisches Institut, Universitat Zurich,
                     Winterthurerstr. 190, CH-8057, Zurich, Switzerland.
SOURCE:              JOURNAL OF MOLECULAR BIOLOGY, (2001 Feb 9) 305 (5) 1111-29.
                     Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY:        England: United Kingdom
                     Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
ENTRY MONTH:         200103
ENTRY DATE:          Entered STN: 20010404
                     Last Updated on STN: 20010404
                     Entered PubMed: 20010222
                     Entered Medline: 20010315
```

AB Fluorescence spectroscopy and 1H/2H-exchange techniques have been applied to characterize the folding of an scFv fragment, derived from the humanized anti-HER2 **antibody** hu4D5-8. A stable intermediate, consisting of a native VL domain and an unfolded VH domain, is populated under equilibrium unfolding conditions. A partially structured intermediate, with 1H/2H-exchange protection significantly less than that of the two isolated domains together, is detectable upon refolding the equilibrium-denatured scFv fragment. This means that the domains in the heterodimer do not fold independently. Rather, they associate prematurely before full 1H/2H-exchange protection can be gained. The formation of the native heterodimer from the non-native intermediate is a slow, cooperative process, which is rate-limited by proline cis/trans-isomerization. Unproductive domain association is also detectable after short-term denaturation, i.e. with the proline residues in native conformation. Only a fraction of the short-term denatured protein folds into the native protein in a fast, proline-independent reaction, because of spontaneous proline cis/trans-reisomerization in the early non-native intermediate. The comparison with the previously studied **antibody** McPC603 has now allowed us to delineate similarities in the refolding pathway of scFv fragments.

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L9   ANSWER 2 OF 20  MEDLINE                          DUPLICATE 1
ACCESSION NUMBER:    2000214282      MEDLINE
DOCUMENT NUMBER:     20214282   PubMed ID: 10752617
TITLE:               Direct evidence by H/D exchange and ESI-MS for transient
                     unproductive domain interaction in the refolding of an
                     antibody scFv fragment.
```

AUTHOR: Jager M; **Pluckthun A**
CORPORATE SOURCE: Biochemisches Institut, Universitit Zurich, Switzerland.
SOURCE: PROTEIN SCIENCE, (2000 Mar) 9 (3) 552-63.
Journal code: BNW; 9211750. ISSN: 0961-8368.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200005
ENTRY DATE: Entered STN: 20000606
Last Updated on STN: 20000606
Entered Medline: 20000519

AB The refolding kinetics of a single-chain Fv (scFv) fragment, derived from a stabilized mutant of the phosphorylcholine binding **antibody** McPC603, was investigated by H/D exchange and ESI-MS and compared with the folding kinetics of its constituting domains V(H) and V(L). Both V(H) and V(L) adopt essentially native-like exchange protection within the dead time of the manual-mixing H/D exchange experiment (10 s) and in the case of V(L), which contains two cis-prolines in the native conformation, this fast protection is independent of proline cis/trans isomerization. At the earliest time point resolvable by manual mixing, fewer deuterons are protected in the scFv fragment than in the two isolated domains together, despite the fact that the scFv fragment is significantly more stable than V(L) and V(H). Full H/D exchange protection in the scFv fragment is gained on a time scale of minutes. This means that the domains in the scFv fragment do not refold independently. Rather, they associate prematurely and in nonnative form, a kinetic trap. Unproductive domain association is observed both after equilibrium- and short-term denaturation. For the equilibrium-denatured scFv fragment, whose native structure formation is dependent on a cis conformation of an interface proline in V(L), this cis/trans isomerization reaction proceeds about one order in magnitude more slowly than the escape from the trap to a conformation where full H/D exchange protection is already achieved. We interpret these data in terms of a general kinetic scheme involving intermediates with and without domain association.

L9 ANSWER 3 OF 20 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1999457490 MEDLINE
DOCUMENT NUMBER: 99457490 PubMed ID: 10525411
TITLE: Insight into odorant perception: the crystal structure and binding characteristics of **antibody** fragments directed against the musk odorant traseolide.
AUTHOR: Langedijk A C; Spinelli S; Anguille C; Hermans P; Nederlof J; Butenandt J; **Honegger A**; Cambillau C; **Pluckthun A**
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Winterthurerstrasse 190, Zurich, CH-8057, Switzerland.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1999 Oct 1) 292 (4) 855-69.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1C12
ENTRY MONTH: 199911
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991104

AB Monoclonal **antibodies** were elicited against the small **hydrophobic** hapten traseolide, a commercially available musk fragrance. **Antibody** variable region sequences were found to belong to different sequence groups, and the binding characteristics of the corresponding **antibody** fragments were investigated. The **antibodies** M02/01/01 and M02/05/01 are highly homologous and differ in the binding pocket only at position H93. M02/05/01 (H93 Val) binds the hapten traseolide about 75-fold better than M02/01/01 (H93 Ala). A traseolide analog, missing only one methyl group, does not have the characteristic musk odorant fragrance. The **antibody** M02/05/01 binds this hapten analog about tenfold less tightly than the original traseolide hapten, and mimics the odorant receptor in this respect, while the **antibody** M02/01/01 does not distinguish between the analog and traseolide. To elucidate the structural basis for the fine specificity of binding, we determined the crystal structure of the Fab fragment of M02/05/01 complexed with the hapten at 2.6 A resolution. The crystal structure showed that only van der Waals interactions are involved in binding. The somatic Ala H93 Val mutation in M02/05/01 fills up an empty cavity in the binding pocket. This leads to an increase in binding energy and to the ability to discriminate between the hapten traseolide and its derivatives. The structural understanding of odorant specificity in an **antibody** gives insight in the physical principles on how specificity for such hydrophobic molecules may be achieved.

L9 ANSWER 4 OF 20 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 1999321993 MEDLINE
DOCUMENT NUMBER: 99321993 PubMed ID: 10390351
TITLE: Removal of the conserved disulfide bridges from the scFv fragment of an **antibody**: effects on folding kinetics and aggregation.
AUTHOR: Ramm K; Gehrig P; **Pluckthun A**
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Winterthurerstr. 190, Zurich, CH-8057, Switzerland.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1999 Jul 9) 290 (2) 535-46.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199907
ENTRY DATE: Entered STN: 19990816
Last Updated on STN: 19990816
Entered Medline: 19990730

AB Fluorescence measurements and H/2H exchange experiments monitored by mass spectrometry have been applied to investigate the influence of the conserved disulfide bridges on the folding behavior and in vitro aggregation properties of the scFv fragment of the **antibody** hu4D5-8. A set of four proteins, carrying none, one, or both of the disulfide bridges have been compared regarding their stabilities, folding kinetics and tendency to aggregate. The results show that refolding of all four scFvs is ultimately limited by a slow proline isomerization in the VLdomain, since the native cis -conformation of proline L95 seems to be a prerequisite for formation of the native interface. Starting from short-term denatured protein, with the proline residues in their native conformation, a kinetically trapped intermediate is populated depending on

the conditions, whose rate of conversion is slower than that of the fast-folding molecules. According to deuteron protection patterns determined by mass spectrometry, those domains retaining the disulfide bridge are able to form stable native-like structure, independent of native interface formation. The disulfide-free domains, in contrast, require the native interface for sufficient stabilization. The resistance of the scFvs towards aggregation seems to be critically dependent on the presence of the disulfide bridge in the VHdomain, and thus on the ability of the VHdomain to form stable structure prior to interaction with the VLdomain. The presence of a stable VLdomain in combination with a disulfide-free VHdomain appears to further promote aggregation, indicating the involvement of structured domains in the aggregates.

L9 ANSWER 5 OF 20 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:449739 CAPLUS
DOCUMENT NUMBER: 132:90223
TITLE: SPM for functional identification of individual biomolecules
AUTHOR(S): Ros, Robert; Schwesinger, Falk; Padeste, Celestino; **Pluckthun, Andreas**; Anselmetti, Dario; Guentherodt, Hans-Joachim; Tiefenauer, Louis
CORPORATE SOURCE: Molecular Nanotechnology, Paul Scherrer Institute, Villigen, Switz.
SOURCE: Proc. SPIE-Int. Soc. Opt. Eng. (1999), 3607(Scanning and Force Microscopies for Biomedical Applications), 84-89
CODEN: PSISDG; ISSN: 0277-786X
PUBLISHER: SPIE-The International Society for Optical Engineering
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The identification of specific binding mols. is of increasing interest in the context of drug development based on combinatorial libraries. Scanning Probe Microscopy (SPM) is the method of choice to image and probe individual biomols. on a surface. Functional identification of biomols. is a first step towards screening on a single mol. level. As a model system we use recombinant single-chain Fv fragment (scFv) **antibody** mols. directed against the antigen fluorescein. The scFv's are covalently immobilized on a flat gold surface via the C-terminal cysteine, resulting in a high accessibility of the binding site. The antigen is immobilized covalently via a long **hydrophilic** spacer to the silicon nitride SPM-tip. This arrangement allows a direct measurement of binding forces. Thus, closely related **antibody** mols. differing in only one amino acid at their binding site could be distinguished. A novel SPM-software has been developed which combines imaging, force spectroscopic modes, and online anal. This is a major prerequisite for future screening methods.
REFERENCE COUNT: 18
REFERENCE(S): (1) Allen, S; Biochemistry 1997, V36, P7457 CAPLUS
(2) Anselmetti, D; J Vac Sci Technol B 1994, V12, P1500 CAPLUS
(4) Chilkoti, A; Biophys J 1995, V69, P2125 CAPLUS
(5) Dammer, U; Biophys J 1996, V70, P2437 CAPLUS
(7) Florin, E; Science 1994, V264, P415 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 6 OF 20 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:71159 CAPLUS
DOCUMENT NUMBER: 128:139760
TITLE: Immunoglobulin superfamily domains and fragments with increased solubility
INVENTOR(S): **Pluckthun, Andreas**; **Nieba, Lars**; **Honegger, Annemarie**
PATENT ASSIGNEE(S): Morphosys Gesellschaft Fur Proteinoptimierung M.b.H., Germany; Pluckthun, Andreas; Nieba, Lars; Honegger, Annemarie
SOURCE: PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9802462	A1	19980122	WO 1997-EP3792	19970716
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 938506	A1	19990901	EP 1997-934467	19970716
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000516452	T2	20001212	JP 1998-505618	19970716
PRIORITY APPLN. INFO.:			EP 1996-111441 A	19960716
			WO 1997-EP3792 W	19970716

AB The present invention relates to the modification of Ig superfamily (IgSF) domains, IgSF fragments and fusion proteins thereof, esp. to the modification of **antibody** derivs., so as to improve their soly., and hence the yield, and ease of handling. The inventors have found that this can be achieved by making the region which comprises the interface with domains adjoined to said IgSF domain in a larger fragment or a full IgSF protein, and which becomes exposed in the IgSF domain, more **hydrophilic** by modification. The present invention describes DNA sequences encoding modified IgSF domains or fragments and fusion proteins thereof, vectors and hosts contg. these DNA sequences, IgSF domains or fragments or fusion proteins obtainable by expressing said DNA sequences in suitable expression systems, and a method for modifying IgSF domains, so as to improve their soly., expressibility and ease of handling.

L9 ANSWER 7 OF 20 MEDLINE
ACCESSION NUMBER: 1998422265 MEDLINE
DOCUMENT NUMBER: 98422265 PubMed ID: 9748318
TITLE: Mutual stabilization of VL and VH in single-chain **antibody** fragments, investigated with mutants engineered for stability.
AUTHOR: Worn A; **Pluckthun A**
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: BIOCHEMISTRY, (1998 Sep 22) 37 (38) 13120-7.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981029

Last Updated on STN: 2000[illegible]
Entered Medline: 19981022

AB A set of six mutants of the levan binding single-chain Fv (scFv) fragment A48 (ABPC48), which have the identical light chain but differ gradually in the stability of the heavy chain, was generated. This was achieved by introducing one or both of the stabilizing mutations H-K66R and H-N52S into the VH domain of the A48 wild-type protein, which is naturally missing the conserved disulfide bridge in VH, and into the cysteine-restored variant A48cys scFv. The stabilizing effects of these two mutations in VH, which had been selected in the context of a disulfide-free derivative of this scFv fragment [Proba, K., et al. (1998) J. Mol. Biol. 275, 245-253], were found to be additive and transferable to the cysteine-restored variant of the A48 scFv, thereby generating extremely stable VH domains. The equilibrium denaturation of these scFv fragments was compared with the corresponding isolated VL domain and two of the different isolated VH domains. In the scFv fragment, the VL domain was found to be stabilized by a more stable VH domain, and, conversely, the VH domain was stabilized by a more stable VL domain. A folding intermediate with nativelike VH and denatured VL was found at equilibrium, if VH was significantly more stable than VL. In all other cases, a cooperative unfolding of the scFv was observed. We explain this observation with different contributions of intrinsic domain stability and extrinsic stabilization provided by the partner domain in the single-chain **antibodies**.

L9 ANSWER 8 OF 20 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1998409447 MEDLINE
DOCUMENT NUMBER: 98409447 PubMed ID: 9737871
TITLE: Factors influencing the dimer to monomer transition of an **antibody** single-chain Fv fragment.
AUTHOR: Arndt K M; Muller K M; **Pluckthun A**
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: BIOCHEMISTRY, (1998 Sep 15) 37 (37) 12918-26.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199810
ENTRY DATE: Entered STN: 19981029
Last Updated on STN: 19981029
Entered Medline: 19981020

AB **Antibody** single-chain Fv (scFv) fragments are able to form dimers under certain conditions, and the extent of dimerization appears to depend on linker length, **antibody** sequence, and external factors. We analyzed the factors influencing dimer-monomer equilibrium as well as the rate of interconversion, using the scFv McPC603 as a model system. In this molecule, the stability of the VH-VL interaction can be conveniently varied by adjusting the ionic strength (because of its influence on the **hydrophobic** effect), by pH (presumably because of the presence of titratable groups in the interface), and by the presence or absence of the antigen phosphorylcholine, which can be rapidly removed due to its very fast off-rate. It was found that the monomer is the thermodynamically stable form with linkers of 15 and 25 amino acids length under all conditions tested (35 &mgr;M or less). The dimer is initially formed in periplasmic expression, presumably by domain swapping, and can be trapped by all factors which stabilize the VH-VL interface, such as the presence of the antigen, high ionic strength, and pH below 7.5. Under all other conditions, it converts to the monomer. Predominantly monomer is obtained during in vitro folding. Monomer is stabilized against dimerization at very high concentrations by the same factors which stabilize the VH-VL interaction. These results should be helpful in producing molecules with defined oligomerization states.

L9 ANSWER 9 OF 20 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 1998443242 MEDLINE
DOCUMENT NUMBER: 98443242 PubMed ID: 9769213
TITLE: Reproducing the natural evolution of protein structural features with the selectively infective phage (SIP) technology. The kink in the first strand of **antibody** kappa domains.
AUTHOR: Spada S; **Honegger A**; **Pluckthun A**
CORPORATE SOURCE: Biochemisches Institut der Universitat Zurich, Winterthurerstrasse 190, Zurich, CH-8057, Switzerland.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1998 Oct 23) 283 (2) 395-407.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981125

AB The beta-sandwich structure of immunoglobulin variable domains is characterized by a typical kink in the first strand, which allows the first part of the strand to **hydrogen** bond to the outer beta-sheet (away from the VH-VL interface) and the second part to the inner beta-sheet. This kink differs in length and sequence between the Vkappa, Vlambda and VH domains and yet is involved in several almost perfectly conserved interactions with framework residues. We have used the selectively infective phage (SIP) system to select the optimal kink region from several defined libraries, using an anti-hemagglutinin single-chain Fv (scFv) fragment as a model system. Both for the kink with the Vkappa domain length and that with the Vlambda length, a sequence distribution was selected that coincides remarkably well with the sequence distribution of natural **antibodies**. The selected scFv fragments were purified and characterized, and thermodynamic stability was found to be the prime factor responsible for selection. These data show that the SIP technology can be used for optimizing protein structural features by evolutionary approaches.

L9 ANSWER 10 OF 20 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 1998437373 MEDLINE
DOCUMENT NUMBER: 98437373 PubMed ID: 9761676
TITLE: The nature of **antibody** heavy chain residue H6 strongly influences the stability of a VH domain lacking the disulfide bridge.
AUTHOR: Langedijk A C; **Honegger A**; Maat J; Planta R J; van Schaik R C; **Pluckthun A**
CORPORATE SOURCE: Biochemisches Institut Universitat Zurich,

Winterthurerstrasse 190, [redacted]ch, CH-8057, Switzerland.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1998) 283 (1) 95-110.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981105

AB Monoclonal **antibody** mAb 03/01/01, directed against the musk odorant traseolide, carries a serine residue instead of the conserved Cys H92 in the heavy chain variable domain, and is thus lacking the highly conserved disulfide bridge. We investigated the energetic consequence of restoring the disulfide bond and the nature of residue H6 (Glu or Gln), which is poised to interact with Ser H92 in the recombinant scFv fragment obtained from this **antibody**. In the scFv fragment derived from this **antibody**, the stabilizing effect of Gln H6 over Glu was found to be as large as the effect of reintroducing the disulfide bond. We have analyzed the conformation and **hydrogen** bond pattern of Gln H6 and Glu H6 in **antibodies** carrying these residues and suggest mechanisms by which this residue could contribute to VH domain stability. We also show that the unpaired cysteine H22 is buried, and conforms to the expected VH structure. The **antibody** appears to have acquired two somatic mutations (Ser H52 and Arg H66), which had been previously characterized as having a positive effect on VH stability. The overall domain stability is the decisive factor for generating functional, disulfide-free **antibody** domains, and several key residues play dominant roles.

L9 ANSWER 11 OF 20 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 1998161424 MEDLINE
DOCUMENT NUMBER: 98161424 PubMed ID: 9502319
TITLE: Parallel pathways in the folding of a short-term denatured scFv fragment of an **antibody**.
AUTHOR: Freund C; Gehrig P; Baici A; Holak T A; **Pluckthun A**
CORPORATE SOURCE: Department of Biochemistry, University of Zurich, Switzerland.
SOURCE: FOLDING AND DESIGN, (1998) 3 (1) 39-49.
Journal code: CUD; 9604387. ISSN: 1359-0278.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980416
Last Updated on STN: 19980416
Entered Medline: 19980408

AB BACKGROUND: **Antibodies** are prototypes of multimeric proteins and consist of structurally similar domains. The two variable domains of an **antibody** (VH and VL) interact through a large **hydrophobic** interface and can be expressed as covalently linked single-chain Fv (scFv) fragments. The in vitro folding of scFv fragments after long-term denaturation in guanidinium chloride is known to be slow. In order to delineate the nature of the rate-limiting step, the folding of the scFv fragment of an **antibody** after short-term denaturation has been investigated. RESULTS: Secondary structure formation, measured by H/D-exchange protection, of a mutant scFv fragment of an **antibody** after short incubation in 6 M guanidinium chloride was shown to be multiphasic. NMR analysis shows that an intermediate with significant proton protection is observed within the dead time of the manual mixing experiments. Subsequently, the folding reaction proceeds via a biphasic reaction and mass spectrometry analyses of the exchange experiments confirm the existence of two parallel pathways. In the presence of cyclophilin, however, the faster of the two phases vanishes (when followed by intrinsic tryptophan fluorescence), while the slower phase is not significantly enhanced by equimolar cyclophilin. CONCLUSIONS: The formation of an early intermediate, which shows amide-proton exchange protection, is independent of proline isomerization. Subsequently, a proline cis-trans isomerization reaction in the rapidly formed intermediate, producing 'non-native' isomers, competes with the fast formation of native species. Interface formation in a folding intermediate of the scFv fragment is proposed to prevent the back-isomerization of these prolines from being efficiently catalyzed by cyclophilin.

L9 ANSWER 12 OF 20 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 97337429 MEDLINE
DOCUMENT NUMBER: 97337429 PubMed ID: 9194169
TITLE: Disrupting the **hydrophobic** patches at the **antibody** variable/constant domain interface: improved in vivo folding and physical characterization of an engineered scFv fragment.
AUTHOR: **Nieba L; Honegger A;** Krebber C; **Pluckthun A**
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: PROTEIN ENGINEERING, (1997 Apr) 10 (4) 435-44.
Journal code: PR1; 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970902
Last Updated on STN: 19970902
Entered Medline: 19970818

AB By constructing Fv and single-chain Fv (scFv) fragments of **antibodies**, the variable domains are taken out of their natural context in the Fab fragment, where they are associated with the constant domains of the light (CL) and heavy chain (CH1). As a consequence, all residues of the former variable/constant domain interface become solvent exposed. In an analysis of 30 non-redundant Fab structures it was found that at the former variable/constant domain interface of the Fv fragment the frequency of exposed **hydrophobic** residues is much higher than in the rest of the Fv fragment surface. We investigated the importance of these residues for different properties such as folding in vivo and in vitro, thermodynamic stability, solubility of the native protein and antigen affinity. The experimental model system was the scFv fragment of the anti-fluorescein **antibody** 4-4-20, of which only 2% is native when expressed in the periplasm of Escherichia coli. To improve its in vivo folding, a mutagenesis study of three newly exposed interfacial residues in various combinations was carried out. The

replacement of one of the residues (V84D [redacted] VH) led to a 25-fold increase of the functional periplasmic expression yield of the scFv fragment of the **antibody** 4-4-20. With the purified scFv fragment it was shown that the thermodynamic stability and the antigen binding constant are not influenced by these mutations, but the rate of the thermally induced aggregation reaction is decreased. Only a minor effect on the solubility of the native protein was observed, demonstrating that the mutations prevent aggregation during folding and not of the native protein. Since the construction of all scFv fragments leads to the exposure of these residues at the former variable/constant domain interface, this strategy should be generally applicable for improving the in vivo folding of scFv fragments and, by analogy, also the in vivo folding of other engineered protein domains.

L9 ANSWER 13 OF 20 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 96292376 MEDLINE
DOCUMENT NUMBER: 96292376 PubMed ID: 8679604
TITLE: Folding nuclei of the scFv fragment of an **antibody**.
AUTHOR: Freund C; **Honegger A**; Hunziker P; Holak T A; **Pluckthun A**
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Switzerland.
SOURCE: BIOCHEMISTRY, (1996 Jun 25) 35 (25) 8457-64.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960828
Last Updated on STN: 19970203
Entered Medline: 19960820

AB The folding kinetics of the variable domains of the phosphorylcholine-binding **antibody** McPC603, combined into a scFv fragment [VH-(Gly4Ser)3-VL), were investigated by the use of fluorescence spectroscopy, nuclear magnetic resonance (NMR), and mass spectrometry (MS). All three methods gave evidence for the occurrence of a major kinetic intermediate during the refolding of the denatured, oxidized scFv fragment. This intermediate is formed within the first 30 s of folding and comprises exchange-protected amide protons of **hydrophobic** and aromatic amino acids, most of which are localized within the inner beta-sheet of the V(L) domain. In the subsequent slow step, most of the amide protons become protected with rate constants that are very similar for residues of both domains. These data are in agreement with the MS results, which indicate a cooperative folding event from the intermediate to the native state of the scFv fragment.

L9 ANSWER 14 OF 20 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 95156486 MEDLINE
DOCUMENT NUMBER: 95156486 PubMed ID: 7853401
TITLE: Tetravalent miniantibodies with high avidity assembling in Escherichia coli.
AUTHOR: Pack P; Muller K; Zahn R; **Pluckthun A**
CORPORATE SOURCE: Biochemisches Institut Universität Zurich, Switzerland.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1995 Feb 10) 246 (1) 28-34.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950322
Last Updated on STN: 19970203
Entered Medline: 19950316

AB We have designed tetravalent miniantibodies assembling in the periplasm of Escherichia coli. They are based on single-chain Fv fragments, connected via a flexible hinge to an amphipathic helix which tetramerizes the molecule. The amphipathic helix is derived from the coiled coil helix of the transcription factor GCN4, in which all **hydrophobic** a positions of every heptad repeat have been exchanged to leucine and all d positions to isoleucine. Gel filtration shows tetramer assembly of the miniantibody even at low concentrations. As expected, the functional affinity (avidity) of the tetravalent miniantibody is higher in ELISA and BIAcore measurements than that of the bivalent construct and the gain is dependent on surface epitope density.

L9 ANSWER 15 OF 20 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95071171 EMBASE
DOCUMENT NUMBER: 1995071171
TITLE: Tetravalent miniantibodies with high avidity assembling in Escherichia coli.
AUTHOR: Pack P.; Muller K.; Zahn R.; **Pluckthun A.**
CORPORATE SOURCE: Biochemisches Institut, Universitat Zurich, Winterthurestr 190,CH-8057 Zurich, Switzerland
SOURCE: Journal of Molecular Biology, (1995) 246/6 (28-34).
ISSN: 0022-2836 CODEN: JMOBAK
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB We have designed tetravalent miniantibodies assembling in the periplasm of Escherichia coli. They are based on single-chain Fv fragments, connected via a flexible hinge to an amphipathic helix which tetramerizes the molecule. The amphipathic helix is derived from the coiled coil helix of the transcription factor GCN4, in which all **hydrophobic** a positions of every heptad repeat have been exchanged to leucine and all d positions to isoleucine. Gel filtration shows tetramer assembly of the miniantibody even at low concentrations. As expected, the functional affinity (avidity) of the tetravalent miniantibody is higher in ELISA and BIAcore measurements than that of the bivalent construct and the gain is dependent on surface epitope density.

L9 ANSWER 16 OF 20 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 94376287 MEDLINE
DOCUMENT NUMBER: 94376287 PubMed ID: 7916382
TITLE: Thermodynamic partitioning model for **hydrophobic** binding of polypeptides by GroEL. II. GroEL recognizes thermally unfolded mature beta-lactamase.
AUTHOR: Zahn R; **Pluckthun A**
CORPORATE SOURCE: Protein Engineering Group, Max-Planck-Institut fur Biochemie, Martinsried, Germany.

SOURCE: JOURNAL OF MOLECULAR BIO[redacted] (1994 Sep 16) 242 (2) 165-74.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199410
ENTRY DATE: Entered STN: 19941031
Last Updated on STN: 19970203
Entered Medline: 19941014

AB By thermal equilibrium measurements we found a three-state folding behavior of mature Escherichia coli beta-lactamase TEM2. The thermodynamically stable intermediate H had no enzymatic activity, but a native-like secondary structure. State H was 9 kcal mol-1 less stable than the native state N and 4 kcal mol-1 more stable than the totally unfolded state U, which is consistent with urea equilibrium measurements of mature beta-lactamase measured under similar conditions. Between 38 degrees C and 50 degrees C there was a decrease in the apparent equilibrium constant for dissociation K'D of the complex between GroEL and mature beta-lactamase, at least partially caused by a decrease in the thermodynamic stability of the native form of mature beta-lactamase. GroEL-bound beta-lactamase was released either after addition of ATP, or in the presence of a competing substrate (i.e. a single-chain antibody), or after lowering the temperature. Whereas at 10 degrees C the folding reaction of mature beta-lactamase was rate limiting, at 37 degrees C the release reaction was the rate-determining step for the regain of beta-lactamase activity, consistent with a decrease of the equilibrium constant for dissociation KD of the complex with temperature. A temperature dependent behavior of GroEL was also observed, when measuring the anilinonaphthalene sulfonic acid (ANS) fluorescence of the chaperone. Similar to all other substrate proteins studied so far, the maximal tryptohan fluorescence of GroEL-bound beta-lactamase was observed at 342 nm. Our results are compatible with a hydrophobic binding pocket of GroEL and confirm the suggested thermodynamic partitioning model for hydrophobic binding of polypeptides by GroEL.

L9 ANSWER 17 OF 20 MEDLINE
ACCESSION NUMBER: 92292158 MEDLINE
DOCUMENT NUMBER: 92292158 PubMed ID: 1602480
TITLE: Refined crystal structure of a recombinant immunoglobulin domain and a complementarity-determining region 1-grafted mutant.
AUTHOR: Steipe B; Pluckthun A; Huber R
CORPORATE SOURCE: Abteilung Strukturforschung, Max-Planck-Institut fur Biochemie, Martinsried, Germany.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1992 Jun 5) 225 (3) 739-53.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199207
ENTRY DATE: Entered STN: 19920724
Last Updated on STN: 19980206
Entered Medline: 19920710

AB We report the solution of the crystal structure of a mutant of the immunoglobulin VL domain of the antibody McPC603, in which the complementarity-determining region 1 segment is replaced with that of a different antibody. The wild-type and mutant crystal structures have been refined to a crystallographic R-factor of 14.9% at a nominal resolution of 1.97 A. A detailed description of the structures is given. Crystal packing results in a dimeric association of domains, in a fashion closely resembling that of an Fv fragment. The comparison of this VL domain with the same domain in the Fab fragment of McPC603 shows that the structure of an immunoglobulin VL domain is largely independent of its mode of association, even in places where the inter-subunit contacts are not conserved between VL and VH. In all three complementarity-determining regions we observe conformations that would not have been predicted by the canonical structure hypothesis. Significant differences between the VL domain dimer and the Fab fragment in the third complementarity-determining region show that knowledge of the structure of the dimerization partner and its exact mode of association may be needed to predict the precise conformation of antigen-binding loops.

L9 ANSWER 18 OF 20 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 91175756 MEDLINE
DOCUMENT NUMBER: 91175756 PubMed ID: 1901023
TITLE: Mapping and modification of an antibody hapten binding site: a site-directed mutagenesis study of McPC603.
AUTHOR: Glockshuber R; Stadlmuller J; Pluckthun A
CORPORATE SOURCE: Genzentrum der Universitat Munchen, Max-Planck-Institut fur Biochemie, Martinsried, West Germany.
SOURCE: BIOCHEMISTRY, (1991 Mar 26) 30 (12) 3049-54.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199105
ENTRY DATE: Entered STN: 19910519
Last Updated on STN: 19910519
Entered Medline: 19910501

AB The quantitative contributions of various amino acid residues to hapten binding in the Fv fragment of the antibody McPC603 were investigated by site-directed mutagenesis. The three-dimensional structure of the Fab' fragment of McPC603 is known to atomic resolution. The haptens phosphocholine, choline sulfate, 3-(trimethylammonium)propane-1-sulfonate, 4-(trimethylammonium)butyric acid, and 4-(trimethyl-ammonium)butyric acid methyl ester were tested for binding. It was found that the phosphate group but not the sulfate and sulfonate groups, interacts with the hydroxyl group of Tyr33(h). The required positive charge for the binding of the phosphate must be contributed by Arg52(h); a lysine at this position or an additional positive charge at position 33(h) abolishes the binding to a phosphocholine affinity column. The interaction between Tyr100(l) and Glu35(h) was found to be essential and could not be functionally replaced by any other pair of residues tested. Binding of the quaternary ammonium ion needs a negative charge; it can reside in either Asp97(l) or Asp101(h), but both together prevent binding to the affinity column. These data may serve as the basis for the development of quantitative treatments of antigen-antibody interactions.

L9 ANSWER 19 OF 20 MEDLINE
ACCESSION NUMBER: 92070228 MEDLINE

DOCUMENT NUMBER: 92070228 PubMed ID: 195
TITLE: Catalytic **antibodies**: contributions from engineering and expression in Escherichia coli.
AUTHOR: **Pluckthun** A; Stadlmuller J
CORPORATE SOURCE: Genzentrum, Universitat Munchen, Max-Planck-Institut fur Biochemie, Martinsried, Federal Republic of Germany.
SOURCE: CIBA FOUNDATION SYMPOSIUM, (1991) 159 103-12; discussion 112-7. Ref: 51
Journal code: D7X; 0356636. ISSN: 0300-5208.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199201
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19920124
Entered Medline: 19920109

AB **Antibodies** have been raised against the transition state of many reactions and shown to catalyse the relevant reaction. Their moderate catalytic efficiencies can be increased by protein engineering, if ways can be found to express the engineered **antibody**. We have developed a system by which fully functional Fv and Fab fragments can be expressed in Escherichia coli. The Fv fragment dissociates at low concentrations; we therefore devised methods to stabilize the fragment. We showed that the Fv fragment of the **antibody** McPC603, a phosphorylcholine-binding immunoglobulin A, binds the antigen with the same affinity as does the intact **antibody** isolated from mouse ascites. Phosphorylcholine is an analogue of the transition state for the **hydrolysis** of choline carboxylate ester. The Fv fragment of McPC603 catalysed this **hydrolysis**. Mutational analysis of the residues in the binding site of the **antibody** has shown which are essential for binding and for catalysis, and the importance of charged residues in certain positions. The E. coli expression system combined with protein engineering and screening methods will facilitate understanding of enzyme catalysis and the development of new catalytic **antibodies**
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L9 ANSWER 20 OF 20 MEDLINE
ACCESSION NUMBER: 91264723 MEDLINE
DOCUMENT NUMBER: 91264723 PubMed ID: 2096820
TITLE: Properties of FV and Fab fragments of the **antibody** McPC603 expressed in E. coli.
AUTHOR: **Pluckthun** A; Glockshuber R; Skerra A; Stadmuller J
CORPORATE SOURCE: Genzentrum der Universitat Munchen, Germany.
SOURCE: BEHRING INSTITUTE MITTEILUNGEN, (1990 Dec) (87) 48-55.
Journal code: 9KI; 0367532. ISSN: 0301-0457.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199107
ENTRY DATE: Entered STN: 19910802
Last Updated on STN: 19970203
Entered Medline: 19910717

AB The FV and Fab fragments of the phosphorylcholine binding **antibody** McPC603 were functionally expressed in E. coli. This was achieved by the co-expression and co-secretion of both chains to the periplasm, where correct processing, folding and assembly occurred. Interestingly, the fraction of correctly folded Fab fragment is smaller than that of the Fv fragment in E. coli. The intrinsic hapten binding affinity was shown to be identical for the recombinant FV or Fab fragment, the whole **antibody** and the Fab fragment obtained by proteolysis from the mouse **antibody**. Fluorescence and crosslinking analyses showed that the FV fragment dissociates at high dilution, but that it is stabilized by hapten binding. The recombinant FV fragment was shown to have catalytic activity to **hydrolyze** choline-p-nitrophenyl carbonate and constitutes therefore a promising model system with which the structural requirements of catalytic **antibodies** can be studied by altering the protein itself.

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FILE 'MEDLINE, CAPLUS, EMBASE, BIOSIS' ENTERED AT 07:17:58 ON 11 JUN 2001
L1 505481 S (ANTIBODY OR IMMUNOGLOBULIN) (P) (HYDROPHILICITY OR SOLUBUL?
L2 47308 S L1 (P) (DOMAIN OR FRAGMENT)
L3 7807 S L2 (P) DNA
L4 17 S L3 (P) INTERFACE
L5 6 DUP REM L4 (11 DUPLICATES REMOVED)
L6 651 S PLUCKTHUN A?/AU OR NIEBA L?/AU OR HONEGGER A?/AU
L7 313 S L6 AND ANTIBOD?
L8 46 S L7 AND HYDRO?
L9 20 DUP REM L8 (26 DUPLICATES REMOVED)

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